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Dated 28 October 1999

30 SEP 1998

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Request for grant of a patent

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The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

P21896/CPA/RMC

2. Patent application number

(The Patent Office will fill in this part)

9821170.9

3. Full name, address and postcode of the or of each applicant (underline all surnames)

University of Ulster at Jordanstown
Newtownabbey
CO ANTRIM
BT37 0QB

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

5965918003

4. Title of the invention

"Marker"

5. Name of your agent (if you have one)

Murgitroyd & Company

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

373 Scotland Street
GLASGOW
G5 8QA

Patents ADP number (if you know it)

1198013

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

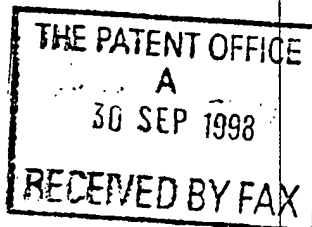
Yes

- a) any applicant named in part 3 is not an inventor, or
 - b) there is an inventor who is not named as an applicant, or
 - c) any named applicant is a corporate body.
- See note (d))

Patents Form 1/77

9. Enter the number of sheets for any following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form



Description

Claim(s)

Abstract

Drawing(s)

6 ✓ NA

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11.

I/We request the grant of a patent on the basis of this application.

Signature *Murgitroyd & Company* Date
Murgitroyd & Company 30 September 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

Roisin McNally, 0141 307 8400

Warning

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Notes

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Patents Form 1/77

1 "Marker"

2

3 The present invention relates to a method of diagnosis
4 of bladder cancer. More particularly the invention
5 relates to an accessible marker for bladder cancer.

6

7 Transitional cell carcinoma of the bladder accounts for
8 1% of all cancers and is the fifth most common
9 malignancy in the over 60s in industrialised parts of
10 the world (Russell et al., 1988; Gleave et al., 1993).
11 Eighty percent of all bladder TCC is superficial at
12 presentation; the remaining 20% is muscle invasive and
13 50% of patients in this category die despite treatment
14 (Simoneau and Jones, 1994). Of those patients
15 initially presenting with superficial tumours, 50 to
16 70% have recurrences within two years. These
17 recurrences are usually superficial although 10 to 20%
18 progress to the muscle invasive form (Farmer et al.,
19 1989; Fradet, 1992; Harland, 1994).

20

21 The high frequency of recurrent TCCB and the increase
22 in disease status in a proportion of patients means
23 that lifetime follow-up using cystoscopy and urinary
24 cytology is essential. The standard procedure is an
25 initial check cystoscopy three months after disease

1 presentation; if this is clear cystoscopy should then
2 be carried out every six months, for one to two years
3 and then annually thereafter with a flexible
4 cystoscope. At present the recurrence rate of TCCB
5 means that annual lifetime cystoscopies should be
6 carried out for all stabilised patients.

7
8 Cystoscopy involves insertion of a cystoscope into the
9 bladder via the urethra to allow visualisation of the
10 tumour using fibre optics. It confirms clinically and
11 pathologically the presence of tumour within the
12 bladder and allows a morphological description (Hossan
13 and Striegall 1993). However it has the disadvantages
14 of being an invasive, uncomfortable procedure. The
15 frequent recurrences of TCCB mean that patients must
16 undergo lifetime follow-up using cystoscopy; this
17 results in the further disadvantage of a large
18 expenditure by the health service.

19
20 Urine cytology is used for the detection of recurrent
21 bladder TCC and although it offers the advantages of
22 being a non-invasive, inexpensive, easily accessible
23 procedure (Zein and Milad, 1991), it has a poor
24 sensitivity, especially at lower stages and grades of
25 disease. The result is false positive and negative
26 findings with reported sensitivities ranging from 37.9%
27 (Miyanaga et al., 1997) to 64% (Martins et al., 1997).

28
29 Numerous studies have been carried out to find the
30 ideal bladder cancer marker. However, none are
31 adequately sensitive or specific enough to fulfil a
32 diagnostic role at present. The most successful to
33 date appears to be the Bard BTA, STAT and TRAK tests
34 with overall sensitivities of 55% (Bard promotional
35 information), 72% (Leyh et al., 1997) and 88% (Bard
36 promotional information) respectively.

1 Bladder cancer is a frequently recurring disease;
2 patients require lifetime monitoring using cystoscopy
3 and urinary cytology. Cystoscopy is an invasive
4 technique and urinary cytology while non-invasive has a
5 low sensitivity.

6
7 It is an aim of the present invention to replace these
8 two procedures with a sensitive, non-invasive urinary
9 test which would allow detection of first presentation
10 and recurrent bladder cancer.

11
12 The invention relates to a 37KDa epidermal growth
13 factor receptor (EGFR) fragment in the urine of
14 patients with transitional cell carcinoma of the
15 bladder (TCCB).

16
17 According to the present invention there is provides a
18 marker for bladder cancer, the marker comprising a
19 37KDa EGFR fragment which is detectable in urine.

20
21 The invention provides a test for the presence of a
22 37KDa EGFR fragment in urine, the test comprising a
23 western blot assay.

24
25 Alternatively the test may comprise an
26 immunochromatographic assay, an ELISA test, latex
27 agglutination or radioimmunoassay.

28
29 The invention further provides a method of diagnosing
30 bladder cancer, the method comprising the steps of
31 reacting a urine sample from an individual to be tested
32 with means to detect a 37KDa EGFR fragment and
33 analysing results.

34
35 Preferably the test is in the form of a dip stick.

36

A 37KDa EGFR fragment has been detected in urine from patients with bladder cancer. First morning urine samples were collected from 24 TCC patients, 6 patients who had bladder cancer previously but who were no disease free and 13 healthy volunteers. 10mls of urine from each was freeze dried and the powdered residue reconstituted in Laemmli lysis buffer. After heating at 110°C for 20 minutes, all samples were stored at - 70°C until required for analysis. Samples were then probed with the Ab4 EGFR antibody (Oncogene Sciences) to the internal domain of the receptor by western blot analysis.

A 37KDa fragment was detected in 88% (21/24) of TCC patients, 66% (4/6) of disease free patients and 7% (1/13) of healthy volunteer urine samples. There was an overall significant association between detection of the 37KDa fragment and presence of bladder cancer. Although four out of six patients who were disease free tested positively, two had benign tumours and two had bladder inflammation at the time the urine sample was taken. This 37KDa fragment therefore appears to be of diagnostic importance. It has a much higher sensitivity than urinary cytology and the Bard BTA and STAT tests, and it appears to be comparable to the Bard TRAK test.

The high frequency of recurrent TCC in the bladder and the progression to a more malignant phenotype in a proportion of patients means that lifetime follow-up using cystoscopy and urinary cytology is essential. Cystoscopy is an evasive procedure and urinary cytology while non-invasive is relatively insensitive. At present the Bard BTA and STAT tests are the only commercially available detectors for bladder cancer. Their sensitivity means that at best they will only act

1 in conjunction with cystoscopy. The Bard TRAK test
2 while more sensitive has yet to be marketed and in fact
3 the results from the present study indicate that the
4 37KDa EGFR fragment is at least comparable. Further
5 work is required to investigate the significance of
6 this fragment in the detection of first presentation
7 and recurrent bladder TCC and to determine whether
8 making it into a quantitative test will offer some
9 insight into prognosis. Appropriate applications are
10 detailed below.

11
12 The 37KDa EGFR fragment may be used as a detector for
13 first presentation bladder and recurrent bladder TCC.
14 Detection of the 37KDa EGFR fragment may be carried out
15 by other methods of investigation as well as western
16 blot analysis. These methods may include
17 immunochromatography, ELISA, latex agglutination or
18 radioimmunoassay. There is currently available a one-
19 step immunochromatographic assay which qualitatively
20 detects bladder tumour antigen in urine in five
21 minutes. Detection of the 37KDa EGFR fragment may be
22 detected by a similar method. Patient urine would be
23 added to the small chamber where it mixes with a
24 colloidal gold-conjugated antibody. If the 37KDa
25 fragment is present, a 37KDa fragment conjugate complex
26 would form. The reaction mixture would flow through
27 the membrane which contains zones of immobilised
28 capture antibodies. In the test zone, the 37KDa
29 fragment conjugate complexes would be captured by a
30 second antigen-specific antibody, forming a visible
31 line. If the 37KDa fragment is not present in the
32 urine, no visible line would form.

33
34 Also or alternatively a dip-stick test may be
35 developed. This may require using methods such as
36 latex agglutination, immunochromatography, ELISA and

1 radioimmunoassay.

2
3 Bladder cancer prognosis has been correlated with a
4 number of factors, the single most important of which
5 is depth of invasion of the bladder wall
6 (Gospodarowicz, 1995); this is followed by grade of
7 tumour (Heney et al., 1983). Other less important
8 factors which influence patient outcome include tumour
9 size (Gospodarowicz, 1995), age of patient at diagnosis
10 (Fitzpatrick and Reda, 1986) and health status
11 (Thrasher et al, 1994). None of these factors can
12 predict prognosis in 100% of patients and so the 37KDa
13 fragment may have some use prognostically. The EGFR
14 fragment may be detected quantitatively using
15 densitometry following western blot analysis and used
16 to predict whether increased levels indicate a better
17 or worse prognosis. Other quantitative methods may be
18 developed to allow easier performance e.g. ELISA or
19 radioimmunoassay techniques.

20
21 EGF and EGFR have been implicated in the pathogenesis
22 of solid tumours such as those of the breast. This
23 simple test developed for urine of patients with
24 suspected TCCb might also be used to identify the
25 diagnostic prognostic role of serum EGFR in other
26 tumour types.
27